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Phospholipid Methylation and the Transmission of Biological Signals Through Membranes

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AXELROD, J. AND F. HIRATA. *Phospholipid methylation and the transmission of biological signals through membranes.* PHARMAC. BIOCHEM. BEHAV. **13**: Suppl. 1, 167–168, 1980.—Enzymatic methylation of phosphotidylethanolamine (PE) to form phophatidylcholine (PC) is associated with translocation of the lipid from the inner cell membrane (PE) to the outer membrane (PC), a concomitant decrease in membrane viscosity, and in some cases, activation of phospholipase A and release of arachidonic acid. Changes in phospholipid methylation are induced by a variety of ligands upon interaction with their specific receptors. In each case stimulation of phospholipid methylation appears to contribute to the propagation of the particular physiological response (e.g., activation of adenylate cyclase in rat reticulocytes; release of histamine by mast cells; chemotactic movement of neutrophils; mitogenesis of lymphocytes). Thus, receptor-mediated changes in phopholipid methylation and membrane fluidity may represent a general mechanism leading to a specific cellular response.

Phospholipid methylation Membrane viscosity Receptors IgE β-Adrenergic agonists
Lymphocytes Neutrophils Mast cells Reticulocytes Phospholipid cascade

RECEPTOR molecules on the surface of cells are the trigger for the transduction of physiological and pharmacological signals initiated by hormones, neurotransmitters and drugs. Recent work in our laboratory has shown that methylated phospholipids and arachidonic acid play an important role in the transmission of biological messages through membranes. We have found that two methyltransferase enzymes are involved in the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) in membranes [6]. These enzymes are asymmetrically distributed in the membrane bilayer. As PE is methylated, it is translocated from the cytoplasmic side of the membrane to form PC, which mainly faces the outer surface of the membrane. Using red cells, we have observed that as phospholipids are methylated the viscosity of the membrane is reduced [5]. In rats that are dependent on alcohol, the membrane viscosity in brain synaptosomes is increased and phospholipid methylation is decreased [2].

Changes in phospholipid methylation in membranes are induced by the interaction of a variety of ligands with their respective receptors. When *beta*-adrenergic receptors in rat reticulocytes are stimulated with *beta*-adrenergic receptor agonists there is an increase in phospholipid methylation, a decrease in membrane viscosity and a generation of cyclic AMP via adenylate cyclase [9]. These results suggest that the interaction of the receptor on the outside of the membrane with a ligand stimulates the methylation and translocation of phospholipids. This increases membrane fluidity, promotes lateral mobility of the receptor and increases the chance for the receptor to collide and couple with adenylate cyclase on the inside of the membrane. An elevated synthesis of phosphatidylcholine via the methylation pathways appears to uncover hidden *beta*-adrenergic receptors in reticulocyte membranes [12], while inhibition of phospholipid methylation reduces the number of *beta*-adrenergic binding sites. Experiments using different ligands (*beta*-adrenergic, benzodiazepine) indicate that phospholipid methyltransferases are clustered around each type of receptor [13]. Binding of ligand with its specific receptor appears to stimulate phospholipid methylation near that receptor and affect membrane changes locally.

Using a variety of cell types such as mast cells, basophils, neutrophils and lymphocytes, a cascade of biochemical changes in membrane lipids leading to a physiological response was observed upon suitable stimulation. Stimulation by specific antigens of IgE receptors on mast cells, for example, results in a release of histamine [11]. Bridging of IgE receptors is necessary for the release of histamine. Stimulation of mast cells with either IgE antigens, antibodies to the IgE receptors or to divalent $F(ab')_2$ fragments, is followed by a transient increase of phospholipid methylation, peaking within 15 seconds [10]. This is followed by an influx of CA^{2+} and histamine release. Inhibition of phospholipid methylation blocks $^{45}Ca^{2+}$ influx and histamine release in

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mast cells. Rat leukemic basophils (RLB) also release histamine upon stimulation of IgE receptors. This cell line has served as a useful model to further characterize the membrane events leading to histamine release. Stimulation of basophils with IgE antigen results in an increased synthesis and metabolism of methylated phospholipids and histamine release [3]. The release of histamine closely parallels the metabolism of methylated phospholipids. Phosphaditylcholine formed by methylation is metabolized by phospholipase A_2 to yield a fatty acid and lysophosphatidylcholine. Treating RLB cells with IgE antigen results in a relase of ^{14}C-arachidonic acid, a fatty acid which was previously incorporated into phosphatidylcholine. Blocking phospholipid methylation inhibits the influx of $^{45}Ca^{2+}$, and release of arachidonic acid and histamine. Inhibiting phospholipase A_2 also prevents arachidonic acid and histamine release. A variety of fatty acids were incorporated into RLB phosphaditylcholine and upon stimulation of these cells with IgE antigen, arachidonic acid was selectively released [1]. All of these observations indicate that stimulation of the IgE receptors results in an increased methylation and translocation of phospholipid, followed by decreased membrane viscosity and an influx of $^{45}Ca^{2+}$. This cation activates phospholipiase A_2 which then hydrolyzes arachidonic acid-rich phosphatidylcholine arising from the methylation pathway, to liberate arachidonic acid and lysophosphatidylcholine. Arachidonic acid is a substrate for two important enzymes, cyclooxygenase which forms prostaglandins, and lipoxygenase which generates hydroxy- and hydroperoxy-lipids. How these compounds then act to liberate histamine remains to be established. Lysophosphatidylcholine is a fusogen and may also be involved in the exocytotic release of histamine.

Receptor-mediated phospholipid methylation was examined in rabbit neutrophils [7]. These cells have receptors for the peptide fMet-Leu-Phe, which upon stimulation causes directed chemotactic movement. The addition of fMet-Leu-Phe to rabbit neutrophils increased the turnover of methylated phospholipids and activated phospholipase A_2, as indicated by the release of ^{14}C-arachidonic acid. Inhibition of phospholipid methyltransferase or phospholipase A_2 blocked the chemotactic response to the chemotactic peptide. Furthermore, stimulation of rabbit neutrophils with the fMet-Leu-Phe resulted in the selective release of arachidonic acid.

Another cell type that utilizes the phospholipid cascade are lymphocytes. Treatment of these cells with certain lectins triggers a series of membrane changes that results in mitogenesis two days later [4]. The lectin, Concanavalin A, causes a transient rise in phospholipid methylation in mouse lymphocytes as well as an increased Ca^{2+} influx and liberation of arachidonic acid from phosphatidylcholine [8]. Forty hours later there is an increased mitogenesis as measured by the incorporation of ^{3}H thymidine into DNA. When phospholipid methylation is inhibited so is Ca^{2+} influx, arachidonic acid release and mitogenesis.

All of these findings indicate that the interaction of several types of cell membrane receptors with agonists results in increased phospholipid methylation and membrane fluidity, activation of the Ca^{2+}-requiring phospholipase A_2, and the formation of arachidonic acid, which leads to a specific cellular response. It is likely that alcohol dependence would affect some of these events.

REFERENCES

1. Crews, F. T., F. Hirata, A. McGivney, J. Axelrod and R. P. Siraganian. Unpublished.
2. Crews, F. T. and E. Majchrowicz. Unpublished.
3. Crews, F. T., Y. Morita, F. Hirata, J. Axelrod and R. P. Siraganian. Phospholipid methylation affects immunoglobin E-mediated histamine and arachidonic acid release in rat leukemic basophils. *Biochem. Biophys. Res. Commun.* **93:** 42–49, 1980.
4. Edelman, G. Surface modulation in cell recognition and cell growth. *Science* **192:** 218–226, 1976.
5. Hirata, F. and J. Axelrod. Enzymatic methylation of phosphadtidylethanolamine increases erythrocyte membrane fluidity. *Nature* **275:** 219–220, 1978.
6. Hirata, F. and J. Axelrod. Enzymatic synthesis and rapid translocation of phosphatidylcholine by two methyltransferases in erythrocyte membranes. *Proc. Natl. Acad. Sci. U.S.A.* **75:** 2348–2352, 1978.
7. Hirata, F., E. Schiffmann, K. Venkatasubramanian, D. Salomon and J. Axelrod. A phospholipase-A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl. Acad. Sci. U.S.A.* **77:** 2533a, 1980.
8. Hirata, F., S. Toyoshima, J. Axelrod and M. J. Waxdal. Phospholipid methylation: A biochemical signal modulating lymphocyte mitogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **77:** 862–865, 1980.
9. Hirata, F., W. J. Strittmatter and J. Axelrod. β-Adrenergic receptor agonists increase phospholipid methylation, membrane fluidity, and β-adrenergic receptor-adenylate cyclase coupling. *Proc. Natl. Acad. Sci. U.S.A.* **76:** 368–372, 1979.
10. Ishizaka, T., F. Hirata, K. Ishizaka and J. Axelrod. Stimulation of phospholipid methylation, Ca^{2+} influx, and histamine release by bridging of IgE receptors on rat mast cells. *Proc. Natl. Acad. Sci. U.S.A.* **77:** 1903a, 1980.
11. Ishizaka, T., K. Ishizaka and H. Tomioka. Release of histamine and slow reacting substance of anaphylaxis (SRS-A) by IgE-anti-IgE reactions on monkey mast cells. *J. Immunol.* **108:** 513–520, 1972.
12. Strittmatter, W. J., F. Hirata and J. Axelrod. Phospholipid methylation unmasks cryptic β-adrenergic receptors in rat reticulocytes. *Science* **204:** 1205–1207, 1979.
13. Strittmatter, W. J., F. Hirata, J. Axelrod, P. Mallorga, J. F. Tallman and R. C. Henneberry. Benzodiazepine and β-adrenergic receptor ligands independently stimulate phospholipid methylation. *Nature* **282:** 857–859, 1979.